659-Pos Board B459
Forward Stepping Mechanism of Kinesin-1 Studied using Asymmetrically-Joined Two-Headed Monomer
Hiroshi Isojima, Michio Tomishige.
Kinesin-1 moves processively along microtubule by alternately moving two motor domains, but the mechanism of the preferential forward stepping is still controversial. The ''neck linker-docking model'' proposes that the neck linker docking of the microtubule-bound head generates forward bias of the tethered head. However, our recent structural analysis of kinesin dimer (Makino et al.) suggested an alternate model in which the tethered head position does not necessarily be biased because the tethered head is not allowed to bind to the rear tubulin-binding site due to a steric constraint on its neck linker and can only release ADP at the forward binding site (''biased-binding model''). To distinguish these mechanisms as alternate steps, we engineered two-headed monomer kinesin by joining two motor heads in tandem on a single polypeptide, in which the neck linker of first head (N-head) is connected to second head (C-head) so that it can propel C-head forward, whereas the neck linker of C-head is free. Single molecule fluorescence observation showed that this two-headed monomer moves processively along microtubules although the velocity was smaller than wild-type dimer by four-fold. In addition, FIONA measurement of individual head showed that both heads takes discrete 16 nm steps, illustrating that this monomer moves by alternately exchanging two heads. Then we measured the dwell time of alternate steps using single molecule FRET and found that forward-stepping of C-head presumably driven by the neck linker docking was less efficient than the forward-stepping of N-head, because the tethered C-head often rebinds to the rear-binding site. These results suggest that biased-binding mechanism is more efficient to drive forward stepping, because rebinding of the tethered head to the rear-binding site is effectively prohibited.

660-Pos Board B460
Ca^{2+} Dependent Dimerization of Kinesin-CaM and Kinesin-M13 Fusion Proteins
Kiyoshi Nakazato, Hideki Shishido, Takeshi Itaba, Kazunori Kondo, Shinsaku Maruta.
Kinesin is known as a dimeric motor protein, which carries cellular cargoes along microtubules by hydrolyzing ATP. Calmodulin (CaM) is a calcium binding protein that participates in cellular regulatory processes. CaM undergoes a conformational change upon binding to calcium, which enables it to bind to specific proteins for a specific response. We have previously demonstrated that kinesin fused with CaM at the C-terminal binds reversibly to M13-Qdots in a calcium dependent manner. In this study, we tried to make the calcium dependent reversible dimerization of kinesin utilizing CaM- target peptide M13 binding system in order to control motility of kinesin. First we designed and prepared the cDNA of the truncated kinesin (355 amino acids) that does not form dimer. We prepared the cDNA encoding two kinesin chimeric proteins in which C-terminal of kinesin355 was fused with calmodulin (K355-CaM) and fused with M13-Cys (K355-M13-cys). The cDNAs of the kinesin chimeras were cloned into expression vector pET21a and transformed into E.coli BL21 (DE3). The kinesin chimeras were successfully expressed and purified by Co-Chelate column. These kinesin chimeras showed normal ATPase activities. Furthermore, K355-CaM bound to M13-YFP in a calcium dependent manner. The calcium dependent interaction between K355-CaM and K355-M13-Cys was examined using Size-exclusion chromatography (SEC)-HPLC, whereas two chimeras did not bind in the absence of Ca^{2+}. In vitro motility assay demonstrated that the dimerized chimera induced microtubule gliding in the presence of Ca^{2+}.

661-Pos Board B461
The Kinesin-1 C-Terminal Tail is Intrinsically Disordered
Mark Seeger, Yongbo Zhang, Sarah Rice.
The C-terminus of kineisn-1 has long been referred to in the literature as the globular tail. In this work we show that this domain is in fact intrinsically disordered. The unfolded structure of the tail domain is revealed via in silico prediction methods, and CD and NMR spectroscopies. It has been well established that a diverse collection of cargos bind exclusively to the tail domain of kinesin-1, and being natively unstructured would allow the tail to sample a variety of conformations in order to accommodate these various binding-partners. Expanding the in silico methods to include other kinesins, we predict that the cargo-binding domains of most members of the human kinesin superfamily are disordered to varying degrees. Therefore, intrinsically disordered sequences may be a general mechanism of cargo binding for many kinesin heavy chains.

662-Pos Board B462
KIF1A Repeats Cycle of 'FREE Diffusion' and 'SPECIFIC Binding' during Weak Binding State
Itsushi Minoura, Masashi Degawa, Rie Ayukawa, Seiichi Uchimura, Ken Sekimoto, **Etsuko Muto**.
The nature of intermolecular interaction between motor and cytoskeletal filament during the weak binding state is not fully understood. In the case of kinesin, while structural analyses revealed that kinesin binds to a specific binding site on tubulin, motility data suggested that kinesin undergoes diffusion, searching for its next binding site. To understand how specific binding and diffusion are compatible in a single ADP state, we analyzed the motion of the single-headed kinesin KIF1A on various mutant microtubules (MTs) in the presence of ADP, using the single molecule motility assay.
We prepared two series of mutant MTs. The first is a series with increased/decreased negative charges at the C-terminal tails (CTTs) of tubulin, reported to be indispensable for the weak binding of KIF1A to the MT (Okada et al., 2000). The second is a series of charged-to-alanine mutants in the H11-12 loop and H12 of tubulin (α-E415, -E416, -E418, -E421 and β-E410, -D417), found to be critical for kinesin motility and ATPase (Uchimura et al., 2010). The analyses of KIF1A movement showed that a reduction of negative charges in CTTs leads to a reduction in both the duration of interaction and the diffusion length of KIF1A, yet the diffusion constant was not greatly changed. In contrast, in most of the charged-to-alanine tubulin mutants, the diffusion constant of KIF1A increased and the duration shortened, but the diffusion length was unaffected. These results indicate that KIF1A-MT interaction in the ADP state can be modeled as an equilibrium between two substates: a dynamic 'diffusion state' and a static 'binding state'. While CTTs stabilize the former, the critical residues in the H11-12 loop and H12 of tubulin stabilize the latter. This model is applicable to dimeric kinesin.

663-Pos Board B463
Mechanochemical Properties of the Kinesin-2 Motor, KIF3A/B, Studied by Optical Trapping
Johan O.L. Andreasson, Bason E. Clancy, William O. Hancock, Steven M. Block.
The kinesin-2 motor KIF3A/B is a processive transport motor that incorporates two different motor domains, coded by separate KIF3A and KIF3B polypeptides. In intraflagellar transport, kinesin-2 motors transport cargo towards the tips of cilia, and dynein motors attached to the cargo are responsible for transport back towards the cell body. While it is known that these opposing motors are responsible for bidirectional transport, little is known about the performance of kinesin-2 motors under load. Here, we used a feedback-controlled optical trap to probe the nanomechanical properties of full-length mouse KIF3A/B under various load regimes and nucleotide concentrations. In addition, each motor domain was characterized by studying mutants consisting of two identical motor domains. Compared to conventional kinesin-1, kinesin-2 velocities were less dependent on load. Moreover, motor processivity, as measured by the run length, depended strongly upon the external load. In a tug-of-war with dynein, such characteristics are expected to enhance the dynamics of directional switching during transport, compared with kinesin-1 which slows under load but remains processive. Experiments using chimeric motors indicate that the load-dependent properties of kinesin-2 are attributable to their motor domains and not, for example, to the lengths of the neck linkers, nor to the properties of the coiled-coil stalks. The velocity data can be modeled in terms of twin alternating three-state cycles, one for each type of motor domain, where ATP binding is followed by a load-dependent transition, presumably neck-linker docking, before hydrolysis. Modeling also suggests that neck-linker docking represents a key mechanochemical step with a shorter characteristic distance for kinesin-2 than kinesin-1. A reduced characteristic distance may facilitate hydrolysis under load and reduce the probability that the tethered motor domain reaches the next microtubule binding site, leading to diminished processivity.

664-Pos Board B464
Neck-Linker-Length Dependence of Processive Kinesin-5 Motility
André Düselder, Christina Thiede, Stefanie Kramer, Christoph F. Schmidt, Stefan Lakämper.
To explore the basic motor activity of the mitotic Kinesin-5, we previously constructed a stable dimeric Kinesin-5 head/Kinesin-1 stalk chimera (Eg5Kin), which contains the motor domain and 14 amino acids of the neck linker of Xenopus leavis Eg5 fused to the neck coiled coil of Drosophila melanogaster Kinesin-1. In contrast to truncated dimeric Eg5-513 (Valentine and Block, 2009, Biophys. J. 97:1671), Eg5Kin is a highly processive motor (Lakämper et al., 2010, J. Mol. Biol. 399:1).

We have here investigated the effect of varying neck-linker length on the motile properties of Eg5Kin. As truncated versions of Eg5 contain the native 18 amino acids of the neck linker, we generated six Eg5Kin constructs comprising of 13 to up to the 18 amino acids of the native Eg5 neck linker, possibly providing a physiological context.
Using single-molecule fluorescence, we found that all six constructs are active motor molecules capable of processive motility. In a first set of experiments, we found that the neck-linker length influences the run length, but not the velocity of the motor. We thus confirm the findings of Shastry and Hancock (2010, Curr. Biol. 20:939) with a different motor. In addition we used optical-trap assays to investigate the change in the average force the motor constructs generated and found only a small variation. Our data thus suggest that the neck-linker length of Eg5 is at least not the sole determinant for speed and force generation.

665-Pos Board B465
Modulation of the Kinesin ATPase Cycle by Neck Linker Docking and Microtubule Binding
Jared C. Cochran, Yu Cheng Zhao, F. Jon Kull.
Kinesin motor proteins use an ATP hydrolysis cycle to perform various functions in eukaryotic cells. Many questions remain about how the kinesin mechanochemical ATPase cycle is fine-tuned for specific work outputs. In this study, we use isothermal titration calorimetry and stopped-flow fluorometry to determine and analyze the thermodynamics of the human kinesin-5 (Eg5/KSP) ATPase cycle. In the absence of microtubules, the binding interactions of kinesin-5 with both ADP product and ATP substrate involve significant enthalpic gains coupled to smaller entropic penalties. However, when the wild-type enzyme is titrated with a non-hydrolyzable ATP analog or the enzyme is mutated such that it is able to bind but not hydrolyze ATP, substrate binding is 10-fold weaker than ADP binding because of a greater entropic penalty due to the structural rearrangements of switch 1, switch 2, and loop L5 on ATP binding. We propose that these rearrangements are reversed upon ATP hydrolysis and phosphate release. In addition, experiments on a truncated kinesin-5 construct reveal that upon nucleotide binding, both the N-terminal cover strand and the neck linker interact to modulate kinesin-5 nucleotide affinity. Moreover, interactions with microtubules significantly weaken the affinity of kinesin-5 for ADP without altering the affinity of the enzyme for ATP in the absence of ATP hydrolysis. Together, these results define the energy landscape of a kinesin ATPase cycle in the absence and presence of microtubules and shed light on the role of molecular motor mechanochemistry in cellular microtubule dynamics.

666-Pos Board B466
Chimeric Kinesin I/Eg5 Constructs Reveal Important Elements to Motor Activity
William R. Hesse, Miriam Steiner, Matthew Wohlever, Roger D. Kamm, Wonmuk Hwang, Matthew J. Lang.
Kinesin I (KHC) and kinesin V (Eg5) have very similar structure, yet very different roles. Eg5 is generally though of as having a low stall force (~1.5pN), slow (unloaded velocity ~100nm s^{-1}), and having limited processivity, while KHC has a stall force of 5-7pN, a velocity of 500-700nm s^{-1}, and having run lengths in the micron range.
We have recently shown that the formation of the cover neck bundle (CNB), which is the formation of a beta sheet between B0 (the coverstrand) and B9 (the first half of the necklinker), is necessary for the motor to generate significant amounts of force. CNB formation along with docking of the necklinker to the motor head (B7) creates the kinesin's power stroke. Loop 13 (L13), which has previously been shown to affect motor velocity with the mutation of highly conserved glycines to alanine, also forms contacts with B9, and has been shown to make a triple beta sheet structure consisting of B0, B9, and L13.
To investigate the relative roles of the coverstrand, B9, and L13 in motor behavior we have created chimeric KHC/Eg5 constructs that incorporate the sequences for these elements from Eg5 into the KHC motorhead. We have found that stall force and unloaded run length are greatly affected by the substitution of Eg5 structural elements into KHC. These results suggest that the motors operate best with a matched CNB and that L13 strongly affects the mechanical strength of the motor. While a match CNB appears to make the relative motor function more robust, B9 has a larger impact on motor function than B0. Furthermore, these structural elements cause the motor to stall at lower forces, be slower, and less processive, but they alone do not turn KHC into Eg5.

667-Pos Board B467
Intra-Motor Domain Coupling is a Strong Driver of Eg5 Motor Activity
Joshua S. Waitzman, Adam G. Larson, Nariman Naber, Eric Landahl, Sarah E. Rice.
The tetrameric kinesin family member Eg5 walks a pair of motor domains along each of two antiparallel microtubules to help set up the mitotic spindle. Previous work has identified a strong coupling between the conformations of two structurally distant elements of the Eg5 motor domain: loop 5 (L5) and the motility-generating neck-linker. However, this work was performed in isolated monomers and how applicable these results are to higher-order assemblies of Eg5 remains unknown. Using electron paramagnetic resonance (EPR) spectroscopy, we have determined the structural relationships between the neck-linkers, L5s and nucleotide binding pockets of isolated Eg5 dimers. Intra-motor domain coupling appears to be a much stronger driver of conformation than inter-motor domain coordination_as was observed in the monomer, the major determinant of neck-linker conformation is the nucleotide state. The docking of the neck-linker upon ejection of ADP is conserved from the monomer to the dimer, and this conformational change is dependent upon the presence of an intact L5. We are currently investigating the effects of disrupting neck-linker-motor domain coupling on dimer motility. Our work forms a basis for future studies of motor domain coordination and provides context for the impact of external regulators on Eg5 activity.

668-Pos Board B468
Coiled-Coil Stalk of Active Kinesin-Like Protein CENP-E is Stably Folded
Nikita Gudimchuk, Yumi Kim, Don W. Cleveland, Fazly I. Ataullakhanov, Ekaterina L. Grishchuk.
CENP-E is a plus-end directed kinesin-like motor, which resides at the kinetochores of mitotic chromosomes. Its activity contributes to chromosome congression in metaphase, and to the capture and stabilization of kinetochore microtubules. Full length CENP-E from Xenopus laevis has a flexible 230nm-long coiled coil stalk, whose function is not known. The stalk is thought to be extended when the motor is active, but it becomes folded in a phosphorylation-dependent manner when the motor domain is inhibited via intramolecular binding to the tail-domain. Here, we investigate the role of this stalk in vitro by studying the motility of microbeads coated with purified recombinant full length CENP-E and a truncated construct, which lacks the stalk and the tail. We show that when conjugated to the beads, both constructs exhibit similar velocities and run lengths even in the absence of in vitro phosphorylation, indicating that the full length CENP-E is entirely active. However, when we used tethered particle motion analysis of the beads carried along microtubules by a single full length motor, we found that CENP-E was not fully extended and behaved as a 40-70nm tether. Phosphorylation of CENP-E with CDK1, Aurora A or MPS1 kinases did not change this length, so this conformation does not depend on phosphorylation. To probe the elastic properties of this compact conformation we applied periodic sideways force to a bead moving along a microtubule. Our measurements demonstrate that the full length CENP-E can be stretched up to 100nm, but it cannot be fully unfolded even by the forces up to 5pN. Together, these data suggest that the compact conformation of CENP-E is stable and phosphorylation-independent, and it does not interfere with normal motor activity.

669-Pos Board B469
Klp Navigation of Spindle Assembly from Poles
Leilani Cruz, Lan Seo, Laura Patrick, **Janet L. Paluh**.
Spindle assembly is the crucial initiating step in the mitotic mechanism. Its formation utilizes coordinated actions of Kinesin-like proteins (Klps) at poles, on microtubules and at chromosomes. We previously identified a novel conserved Kinesin-14 pole-based mechanism for spindle assembly by Klp binding to the γ-tubulin small complex (γ-TuSC) microtubule organizing center, (MTOC). Changes to the γ-TuSC MTOC in fission yeast alter microtubule dynamics to regulate bipolarity and both human and fission yeast Kinesin-14 Klps can regulate this mechanism. The Kinesin-14 Pkl1 motor domain associates with residues in helix 11 of γ-tubulin at a novel Klp binding site. The Kinesin-14 Pkl1 Tail domain is distinct from that of the well-characterized *Drosophila* Ncd, replacing microtubule association sites with specialized elements for spindle pole targeting and regulation of the γ-TuSC. Thus unlike *Drosophila* Ncd that crosslinks microtubules to stabilize mitotic spindle assembly, Kinesin-14 Pkl1 regulates assembly through the γ-TuSC. Klps can be classified into 14 families. Members of two ubiquitous families, Kinesin-14 and opposing Kinesin-5 Klps, each exhibit distinctions at multiple levels amongst members, including domain elements, functional mechanisms and spindle localization.